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## Single-Domain Antibodies: Rugged Recognition Elements

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**Introduction:** The ability to quickly and accurately detect potential bio-threat agents is a priority for the Department of Defense and for homeland security. Most rapid diagnostic and detection immunoassays rely on monoclonal or polyclonal antibodies (IgG) as the recognition elements. Although sensitive and specific, these "conventional" antibodies are time-consuming to develop and have limited stability. In order to form the antigen binding site, conventional IgG require the pairing of variable (V) heavy and light domains. Cloned derivatives of conventional IgG that comprise just these V domains form a minimal antigen binding site. These derivatives have long been used to develop recognition elements for biosensor applications. However, these single-chain antibodies (scFv) are often less stable than the parental full-length antibodies and aggregate irreversibly at elevated temperatures due to their two-domain structure. Ideally, development of a one- or single-domain structure capable of antigen binding may avoid aggregation upon heating and would facilitate the application of biosensors at elevated environmental temperatures or for continuous use over long periods of time.

In the mid 1990s, it was discovered that certain animals, such as camels, llamas, and sharks, possess a class of "unconventional" immunoglobulins consisting of heavy-chain homodimers where antigen binding is mediated through a single V domain (Fig. 4). These V domains, when cloned as single-domain antibodies (sdAb), comprise the smallest known antigen binding fragments (13-15 KDa). SdAb can refold to bind antigen after chemical or heat denaturation, enabling them to retain the ability to bind antigen after exposure to elevated temperatures. Our goal was to develop recombinant libraries of sdAb to permit the selection and evaluation of these unique biomaterials for the benefit of future biodefense requirements.

**Libraries and Selections:** In a collaborative effort, NRL and the Southwest Foundation for Biomedical Research have developed large libraries of sdAb, derived from llama (1 billion representatives) and spiny dogfish shark (60 million representatives), which are displayed on filamentous phage M13. We selected the llama-derived library for binding to a variety of real and surrogate bio-threat agents and then isolated binders in each selection. Llama-derived sdAb were isolated against model protein hen egg lysozyme (HEL), cholera toxin, staphylococcal enterotoxin B (SEB), ricin, and live vaccinia virus (a smallpox surrogate). The spiny dogfish sdAb library was useful for the delivery of cholera toxin-binding sdAb.

**Characterization:** We demonstrated that our isolated binders were specific, binding to cognate target and not irrelevant antigens. We further showed that binding of the anti-toxin sdAb to a toxin-coated surface could be countered by the addition of soluble target. This is important, since it demonstrates that the sdAb can recognize toxin in solution and not merely bind to immobilized antigen, which may be partially denatured.

To test for improved heat stability, isolated sdAb were heated to elevated temperatures for 5 minutes, cooled, and then assayed for target binding and interaction with an irrelevant antigen. These tests were done in parallel with conventional antibodies, and in nearly all cases the sdAb retained specificity and activity upon heating to the highest temperatures (95° to 100 °C) while the conventional antibodies lost their binding ability. As a further test of thermal stability and an indicator of improved shelf life, sdAb were heated to 95 °C for various lengths of time (Fig. 5). Again, these experiments were done side-by-side with conventional antibodies. All examined sdAb proved more stable than conventional antibodies when subjected to prolonged heating. Some retained 100% of their binding ability even after heating to 95 °C for 45 minutes. Other sdAb retained close to 100% binding activity for the first ~10 minutes and then slowly lost activity over the course of 60 to 90 minutes. The majority of conventional antibodies lost ~90% of their binding activity after the first 5 minutes of heating, while the best conventional antibody lasted no more than 20 minutes at 95 °C prior to losing ~90% of its binding activity.

The sandwich assay format employed by many biosensors uses an immobilized antibody together with a labeled antibody to form an antibody-antigen-antibody sandwich. We have demonstrated the ability of our sdAb to be used in this format for the detection of toxins (Fig. 6), demonstrating the applicability of the sdAb for detection and to diagnostics-type assays.

**Conclusions:** Our developed sdAbs represent a new generation of durable detection reagents that can be integrated into any antibody-based biosensor. The libraries we developed are renewable and available to be rapidly mined for recognition elements for current and future bio-threat agents. SdAb will benefit the DoD and homeland security by permitting the fielding of biosensors with rugged recognition elements selective against a wide range of targets.

[Sponsored by DTRA and NRL]

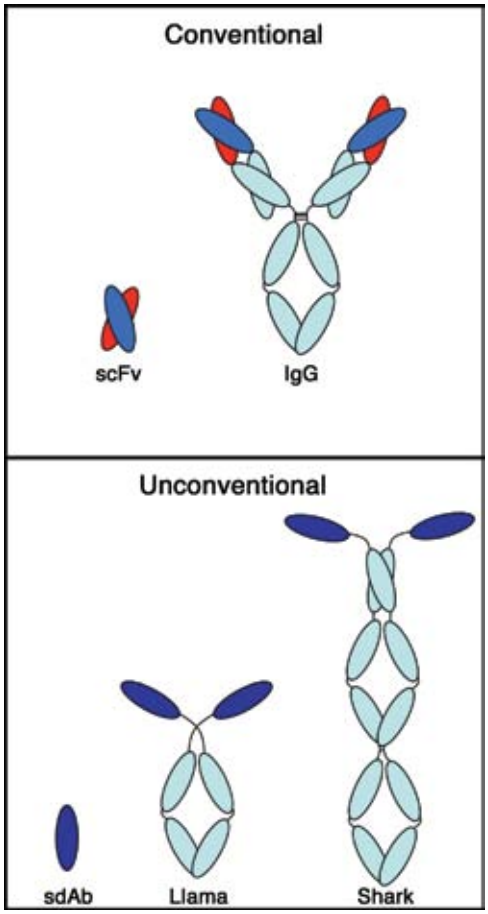


**FIGURE 4**
Schematic representation of (top) a conventional antibody (IgG) and its cloned minimal antigen binding fragment (scFv), and (bottom) shark and llama antibodies and their cloned minimal binding fragment (sdAb).

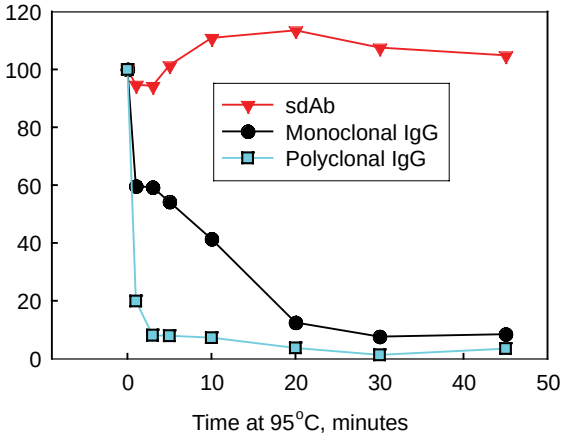


**FIGURE 5**
Thermal stability of anti-ricin reagents; sdAb (red) and conventional monoclonal (black) and polyclonal (blue) antibodies.

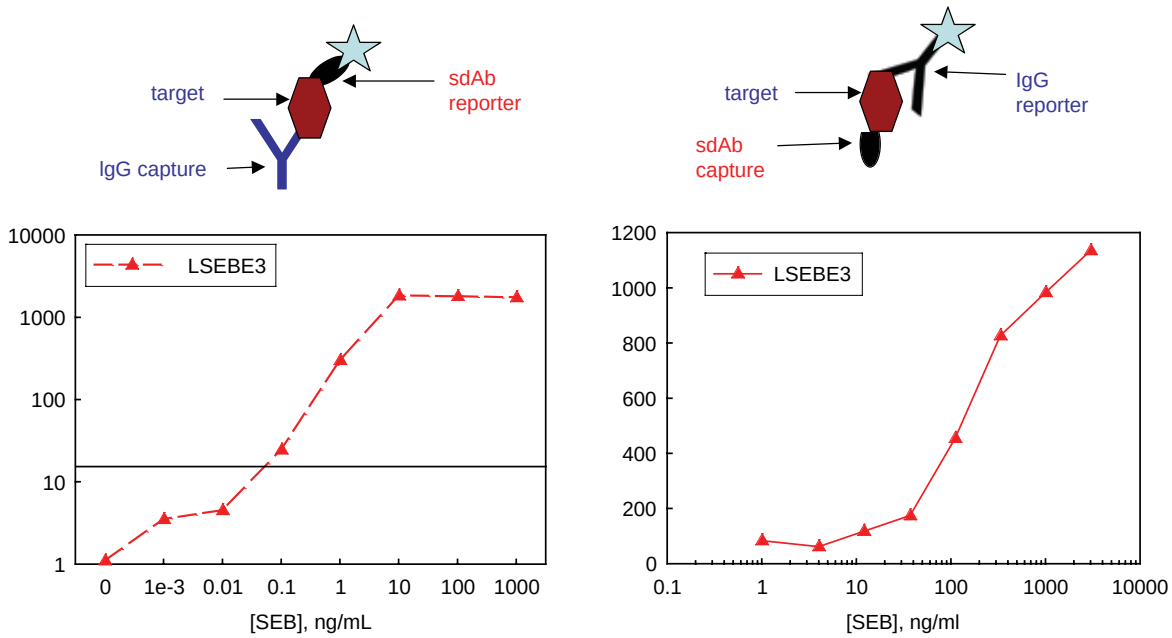


**FIGURE 6**
Performance of anti-SEB sdAb as a reporter (left) and capture (right) in sandwich assays for SEB.